Influenza: Mitigation and Prevention

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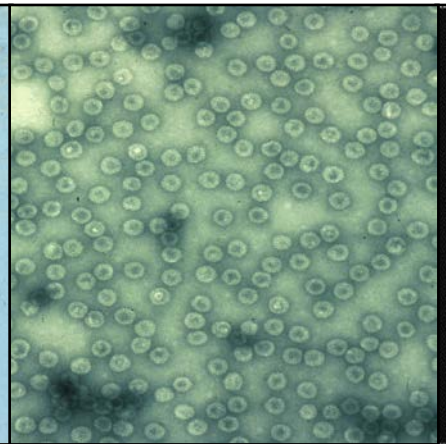
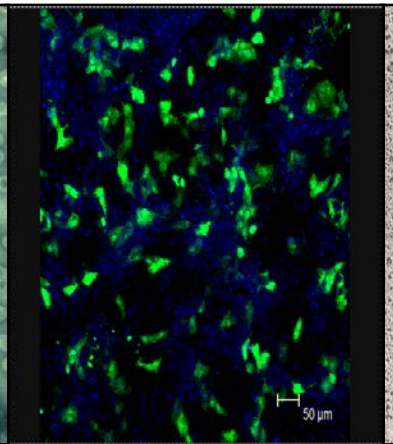
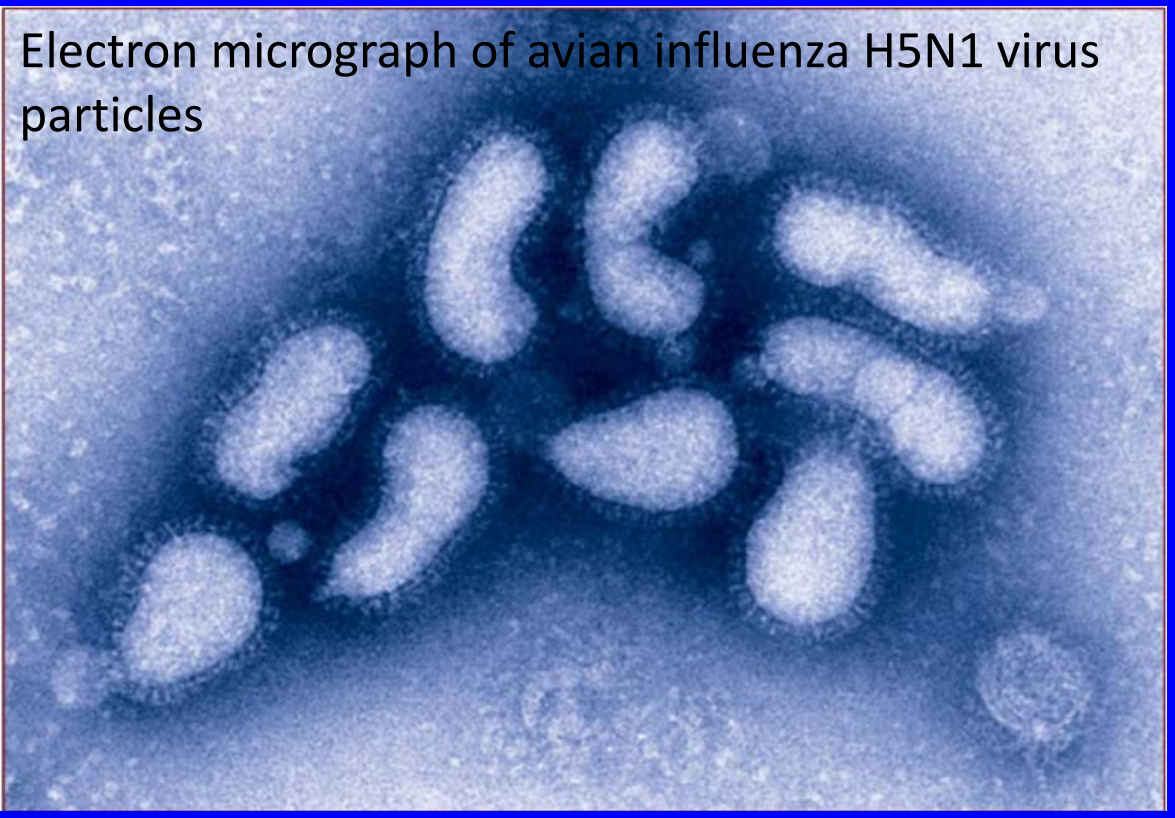




Influenza Virus

Causative agent of influenza


Electron micrograph of avian influenza H5N1 virus particles

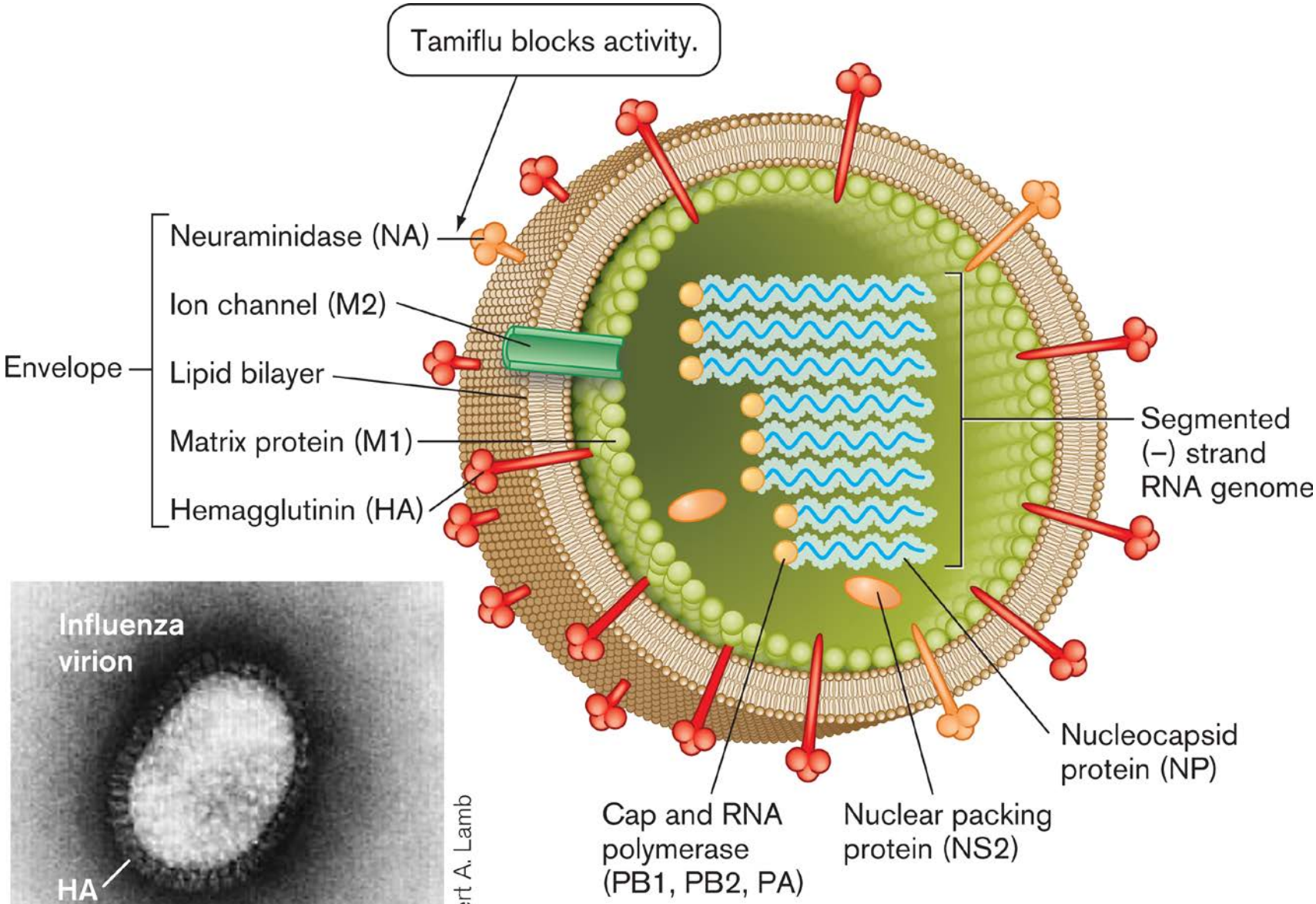
Infection can lead to:

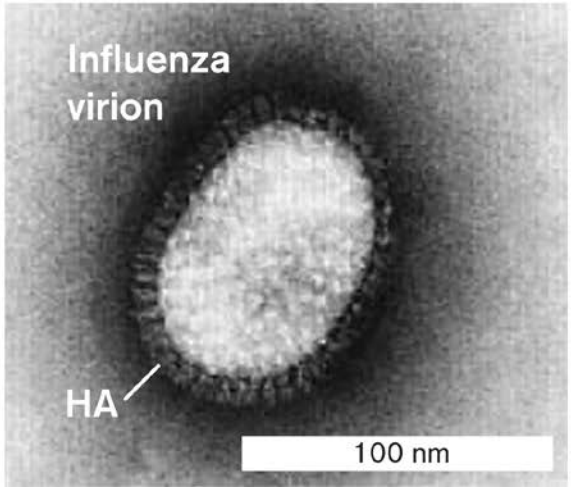
- **pneumonia**
- **sinus/ear infection**
- **worsening of chronic conditions (asthma, diabetes, heart disease)**
- **Death**

Courtesy of R. Fleck, National Institute of Biological Standards and Control, UK

Influenza,
the annual killer epidemic that won't go away…

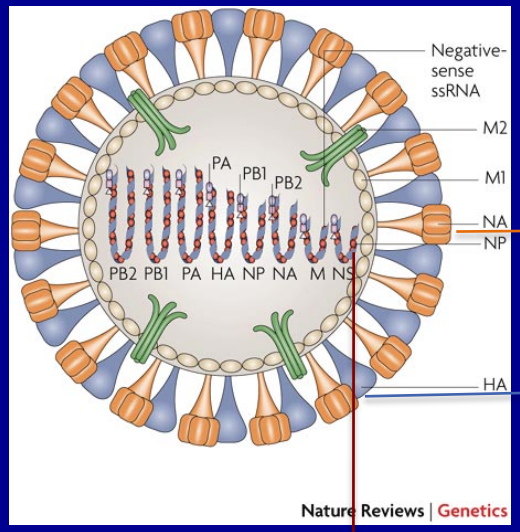
Structure of influenza A





Robert A. Lamb

Influenza Virus Nomenclature



Neuraminidase

Hemagglutinin

Type of Nucleoprotein

A/Fujian/411/2002 (H3N2)

Virus Type

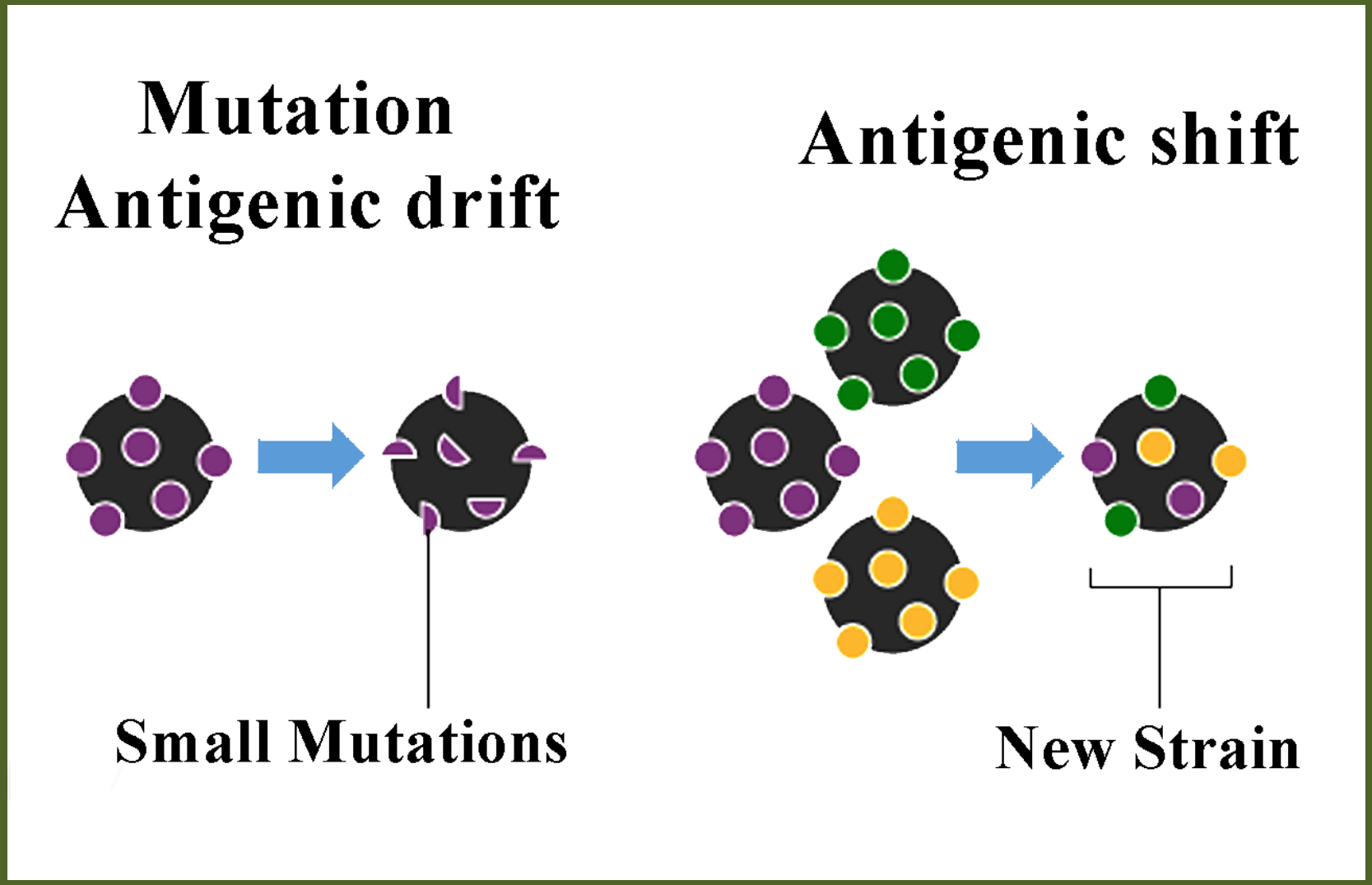
Geographic Origin

Strain Number

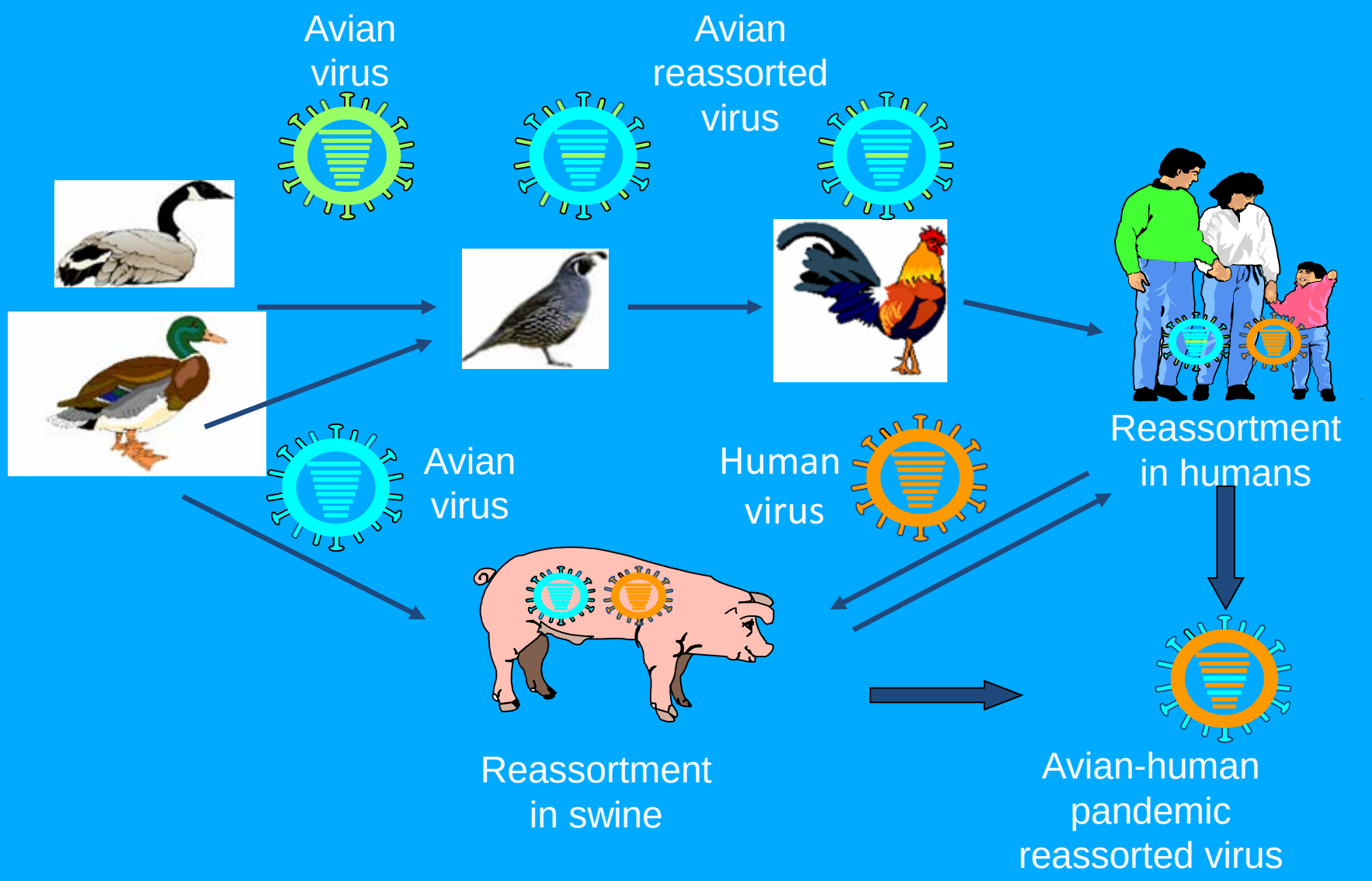
Year of Isolation

Virus Subtype

Antigenic shift and drift



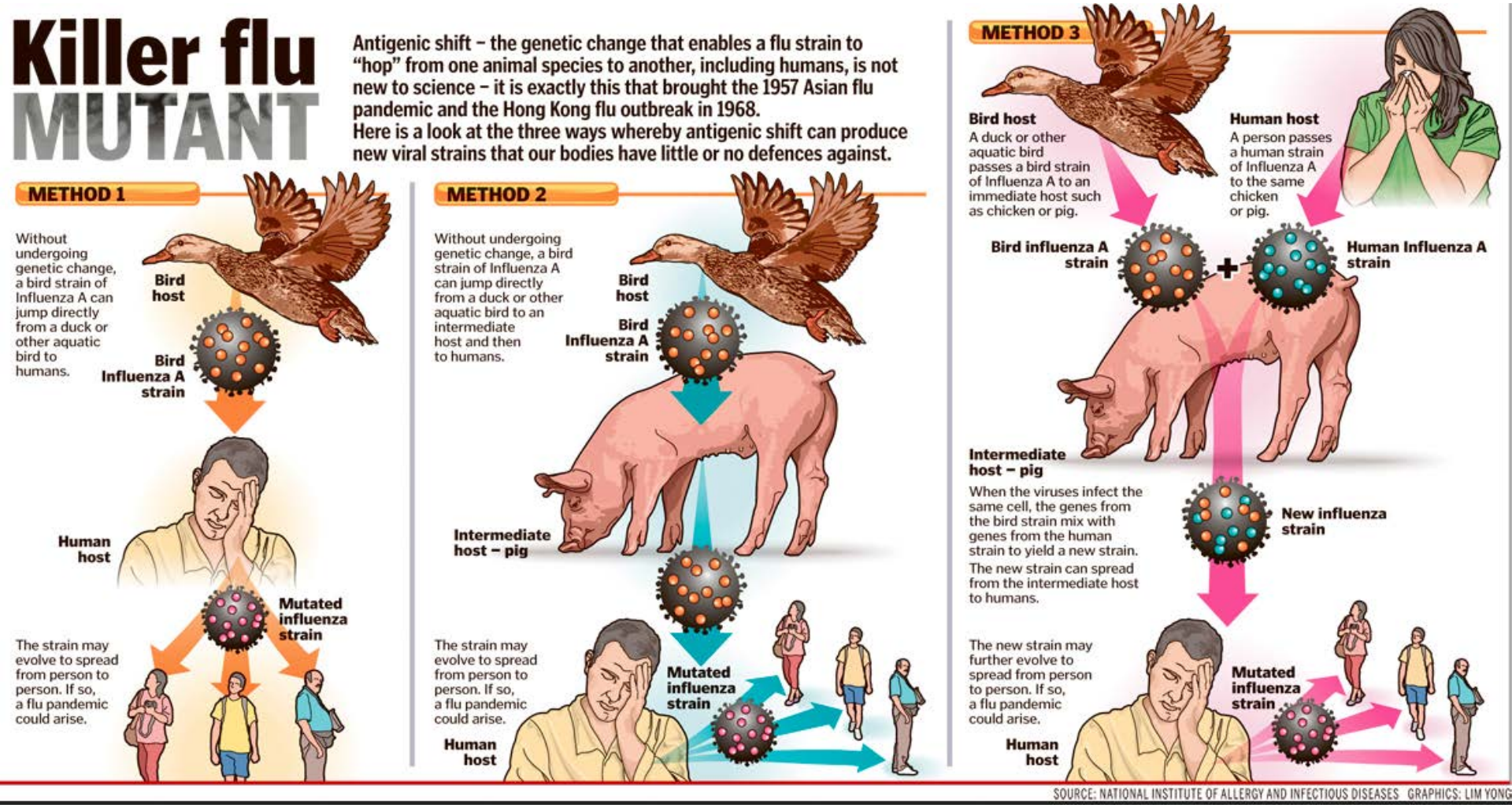
Shift and Pandemic Influenza



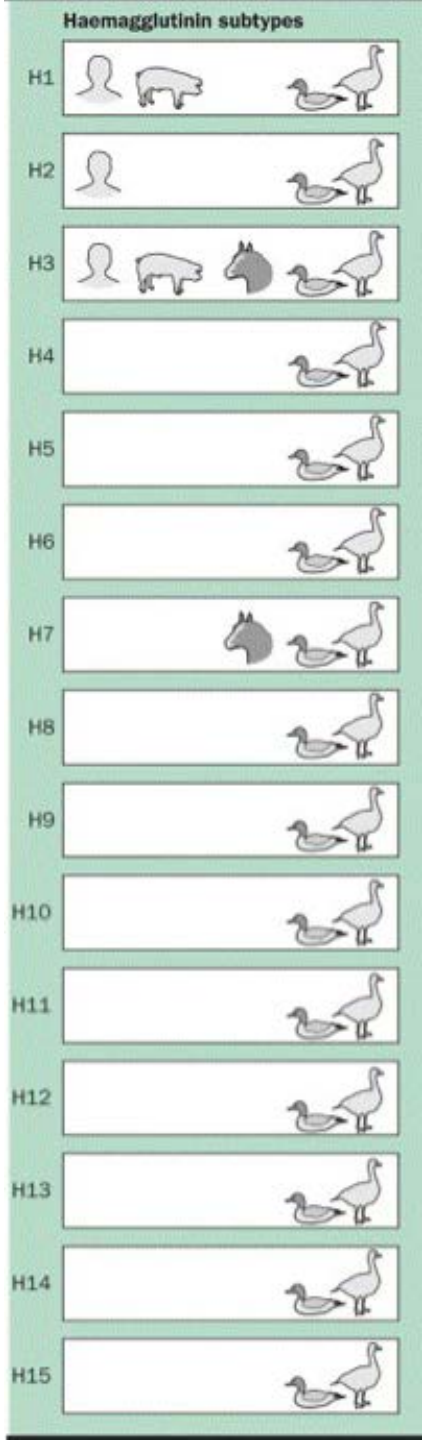
CDC

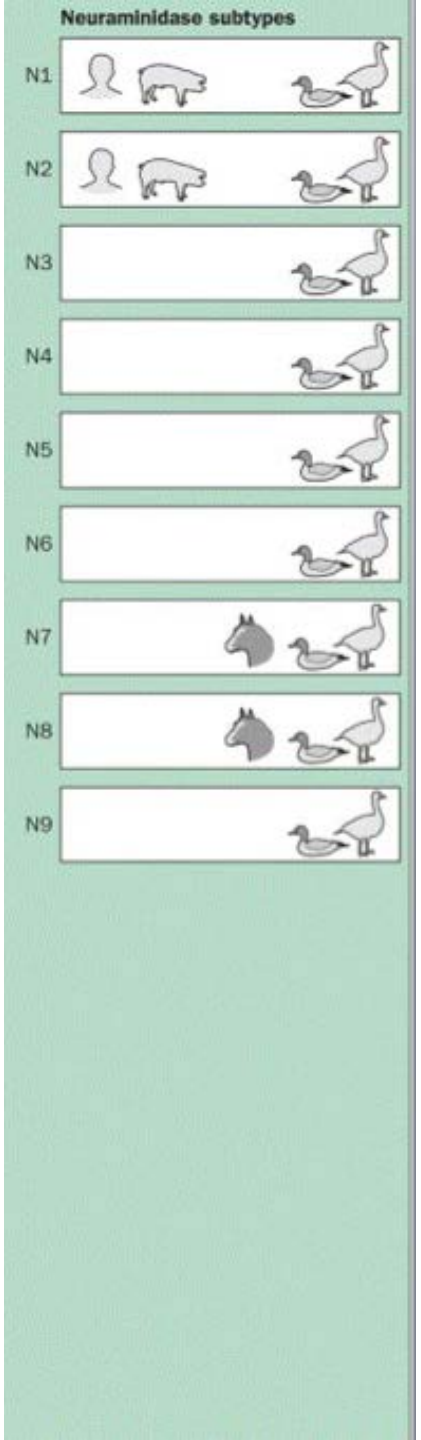
Antigenic shift

- Models for the Generation of Pandemic Influenza Virus



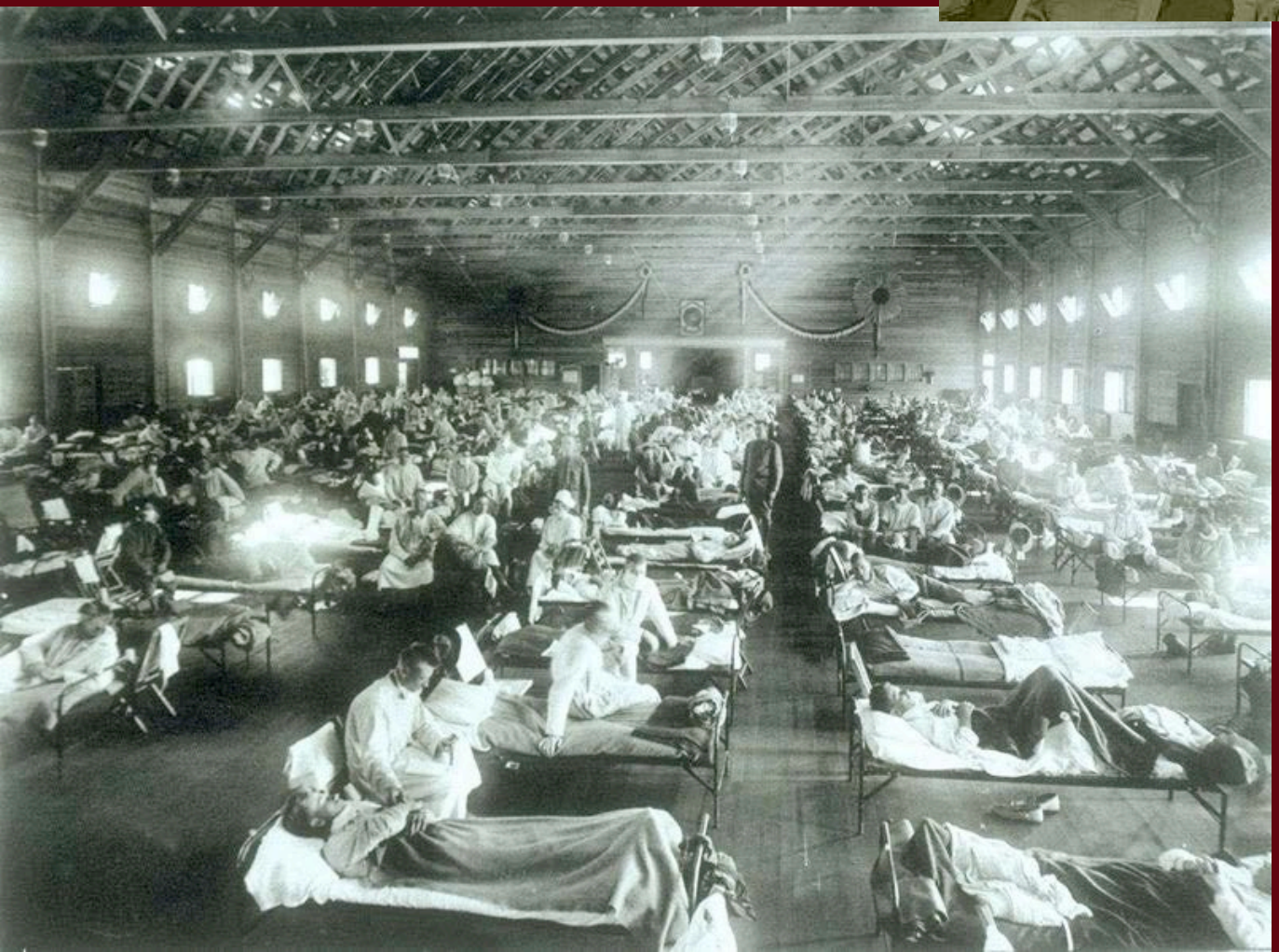
Hemagglutinin and Neuraminidase Subtypes of Influenza A Virus





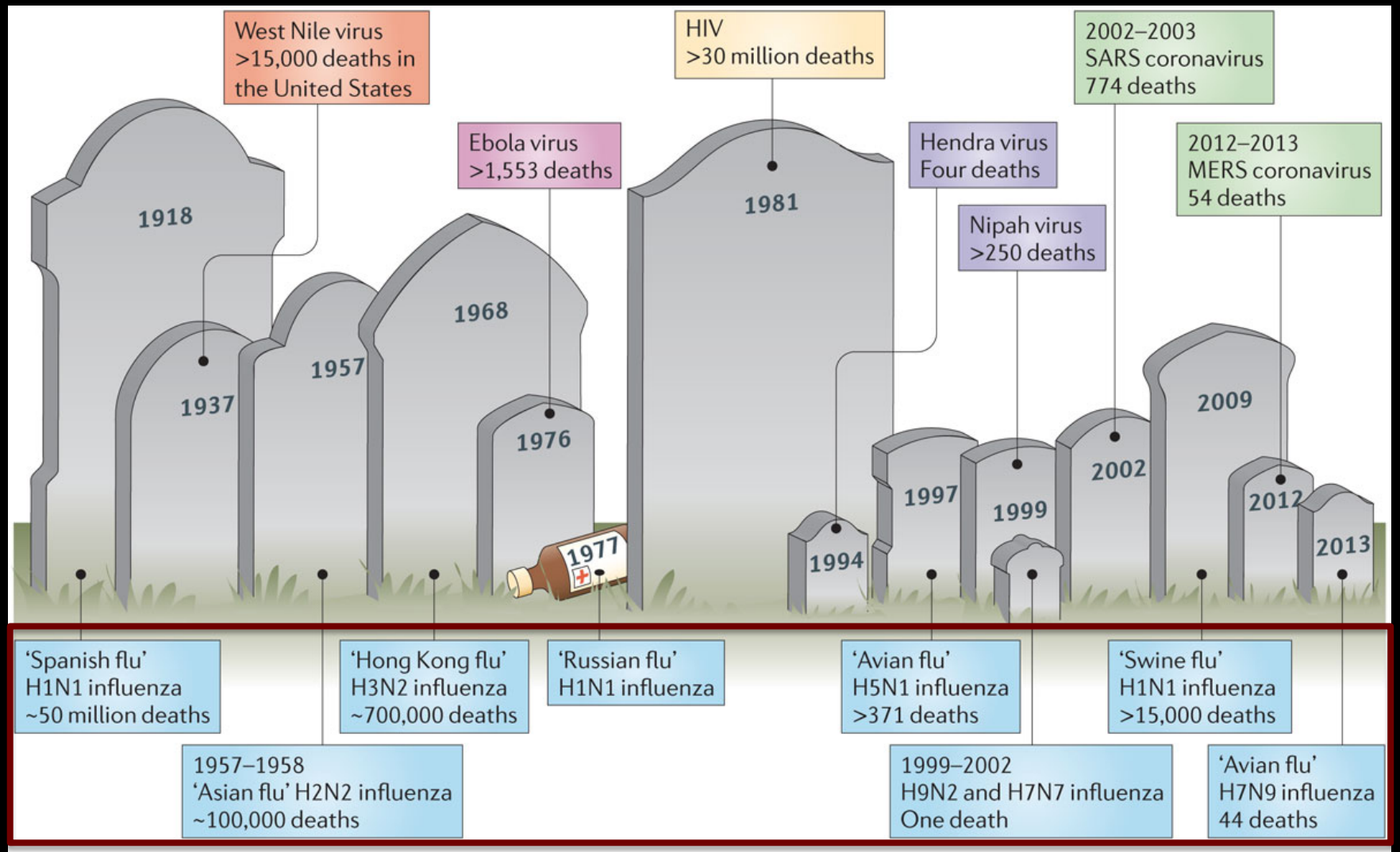
The Great Pandemic

The Spanish flu pandemic of 1918



It infected 500 million, people across the world and killed 50 to 100 million of them - three to five percent of the world's population.

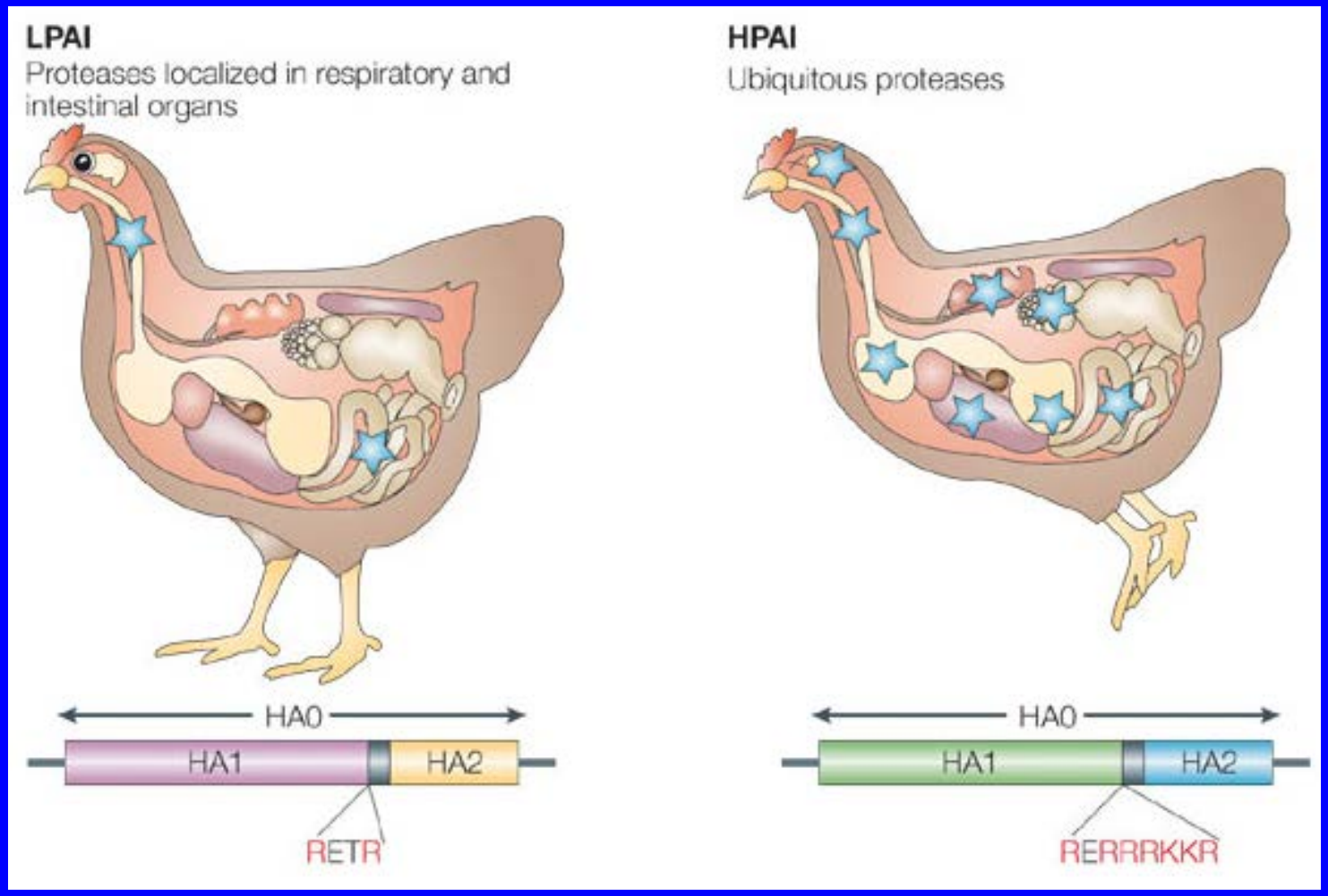
Over the past century, humanity has witnessed numerous emergences of zoonoses - Influenza viruses account for an important proportion of these deaths



Nature Reviews | Immunology

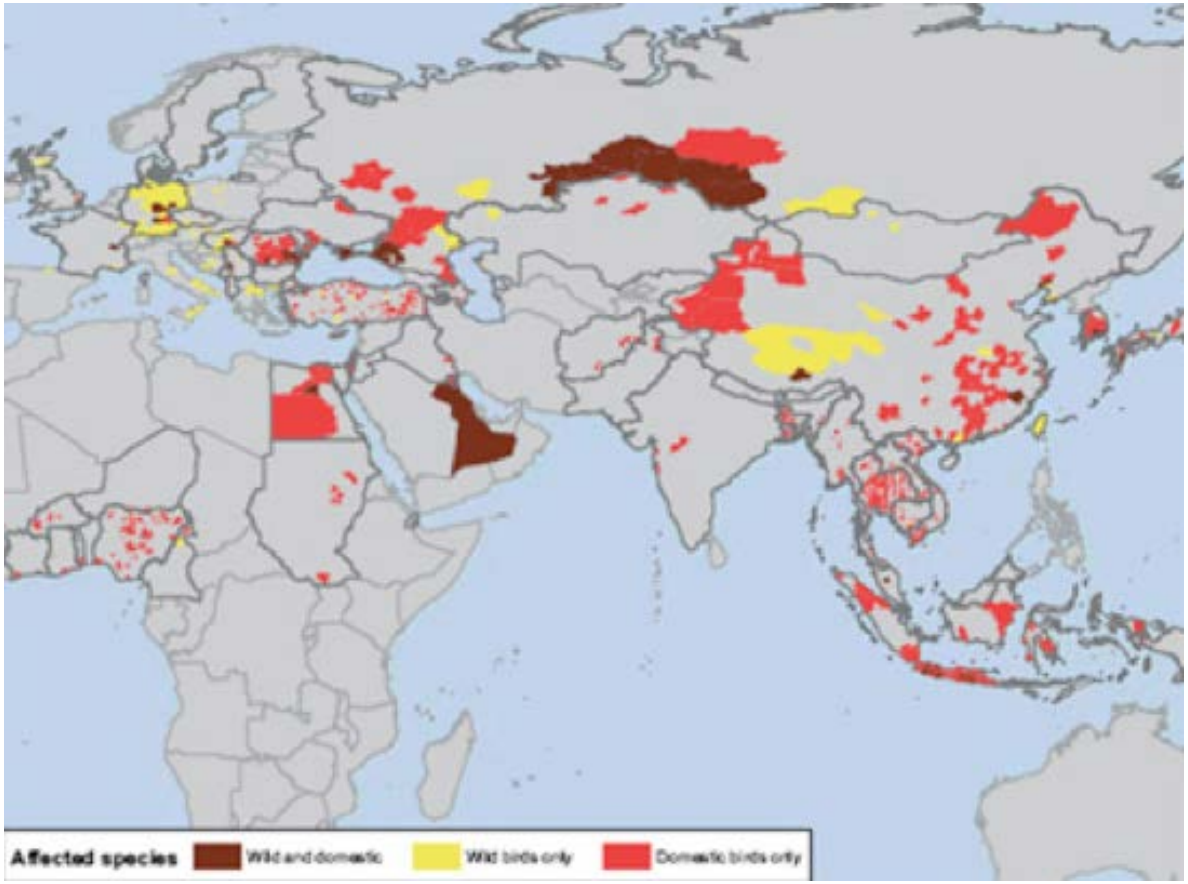
www.timeasia.com
FEBRUARY 9, 2004
TIME
BIRD FLU
Is Asia hatching the next human pandemic?

Haemagglutinin (HA) is a major determinant of the pathogenicity of avian influenza viruses in poultry



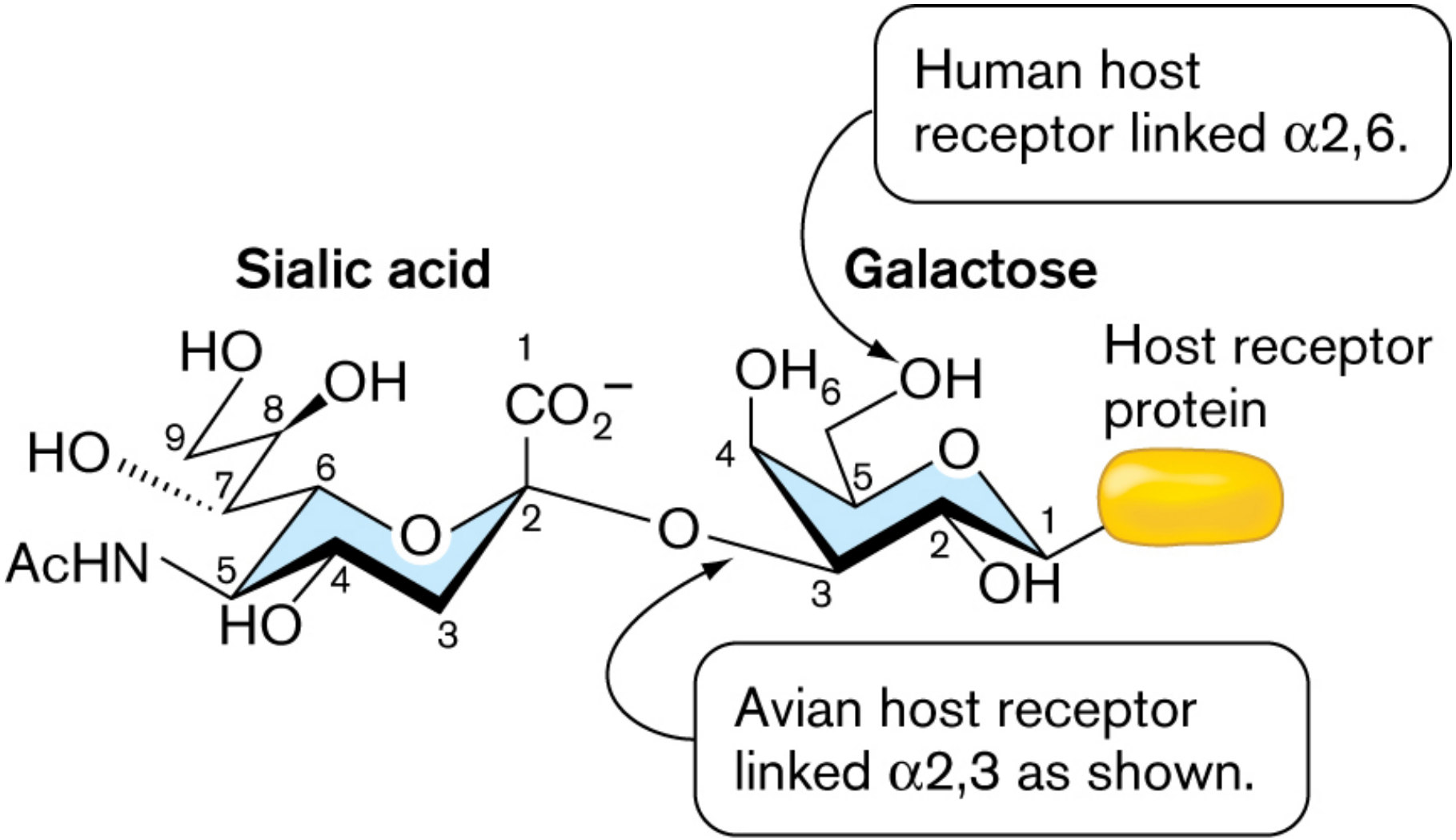
H5N1 SPREADS

Countries in which the H5N1 HPAI virus has been detected in domesticated poultry and migratory wild birds



(Source: Wild birds and Avian Influenza: An Introduction to Applied Field Research and Disease Sampling Techniques. 2007.)

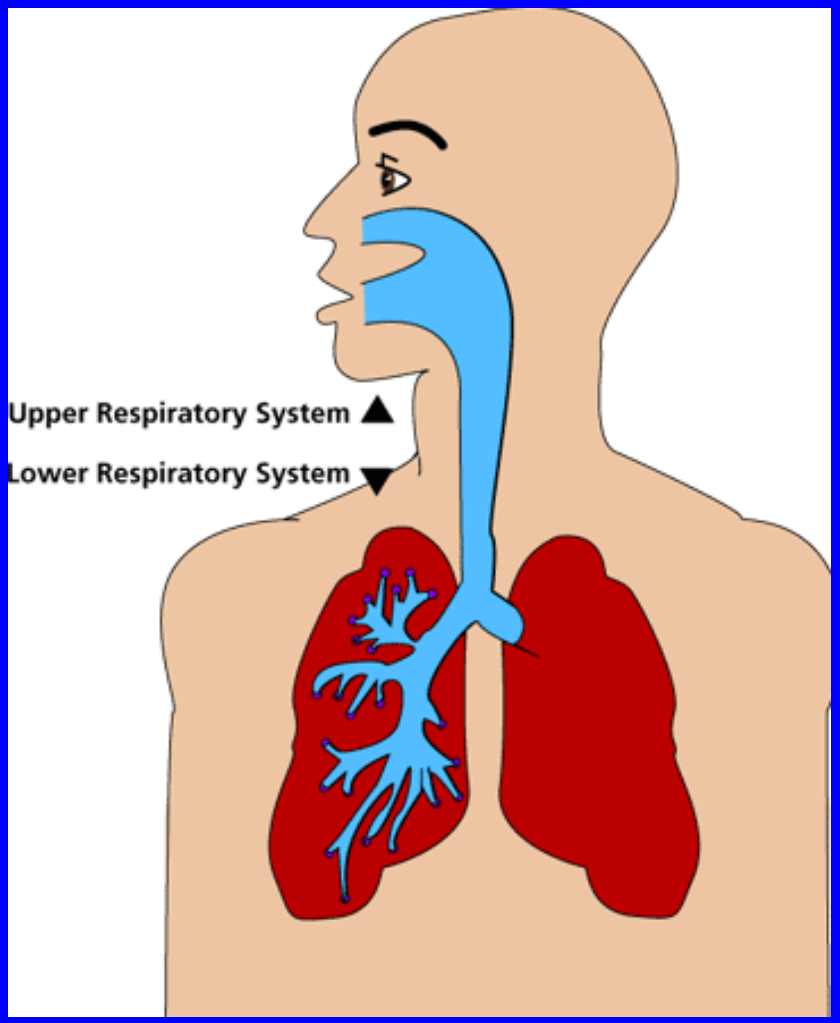
Sialic acid in the influenza receptor



Cell barrier slows bird flu's spread among humans

Why H5N1 viruses rarely infect and spread from human to human?

Cells in the upper portions of the respiratory system lack the surface receptors that enable avian H5N1 virus to dock with the cell.



The H5N1 avian flu virus has infected and killed hundreds of people, despite the fact that, at the moment, the virus can't spread easily between people. It thus only infects those working with infected poultry.

The death toll could become much worse if the virus gains the ability to infect in the human upper respiratory tract and becomes airborne!!!

Therapy/Prevention of Influenza

- Medications
  - Requires use of drugs such as Tamiflu early in the disease course
  - All not uniformly effective against Influenza Types A and B
  - Can be costly
  - Not all approved for children

- Vaccine (s)

- Handwashing!

Standard influenza vaccines:

Trivalent Inactivated Vaccine (TIV), intramuscular.

Influenza Vaccine Virus Strains for 2013-14:

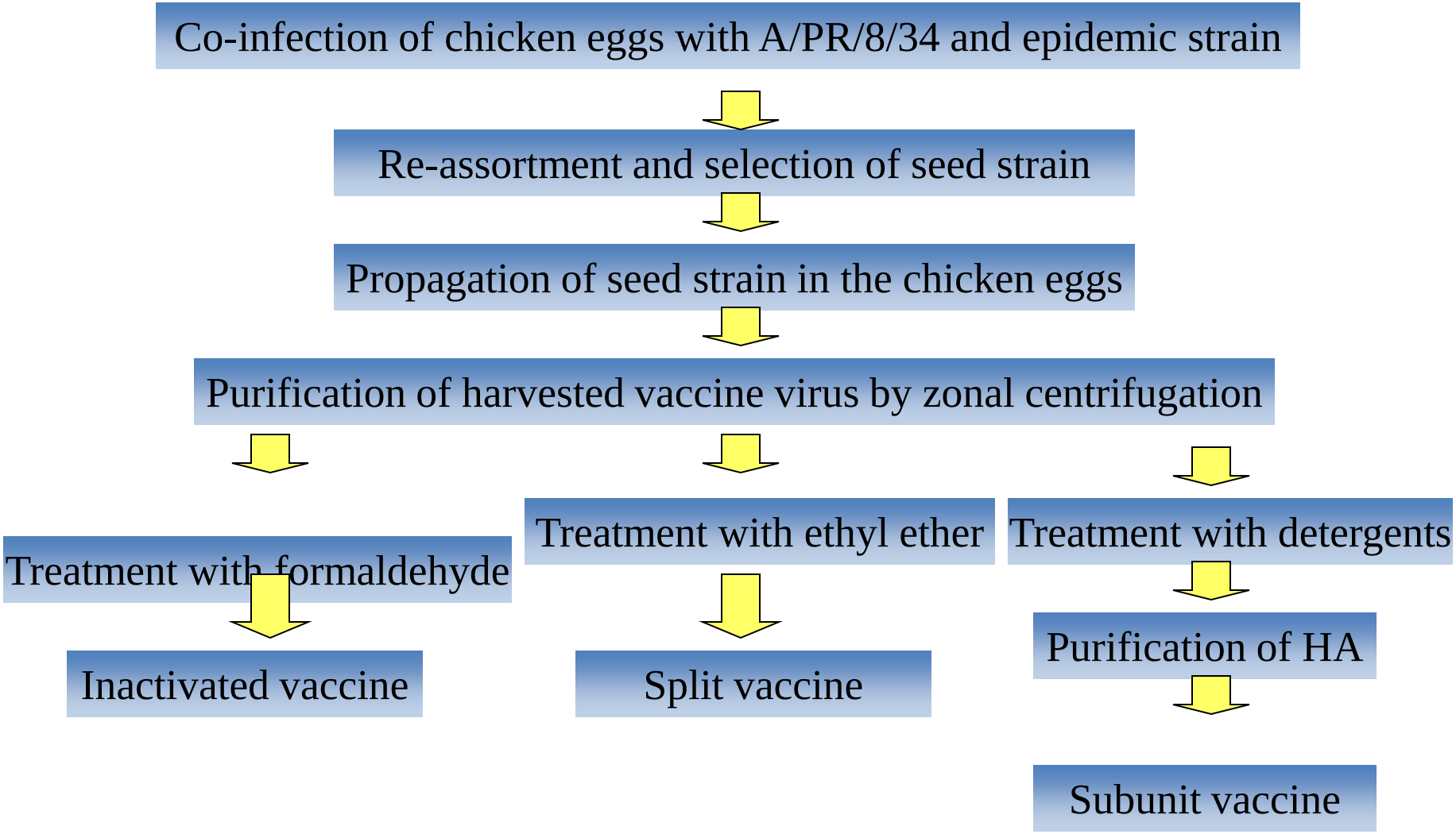
- **Trivalent vaccines will contain:**

  A/California/7/2009 (H1N1)-like virus,
  A/Victoria/361/2011
  B/Massachusetts/2/2012-like virus (Yamagata lineage).

- **Quadrivalent vaccines, will contain, in addition:**

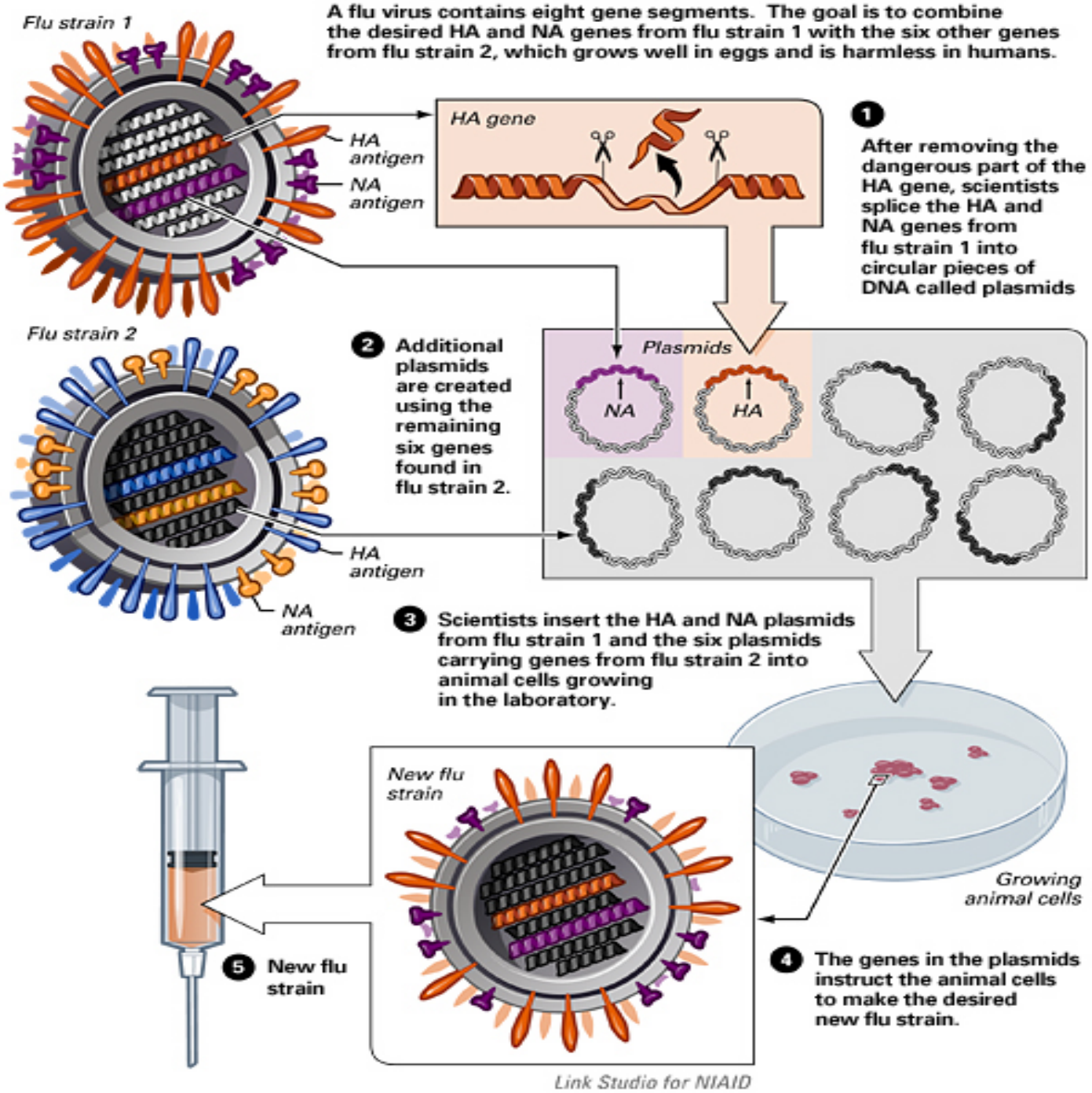
  B/Brisbane/60/2008-like virus (Victoria lineage)

Traditional production of influenza virus vaccine



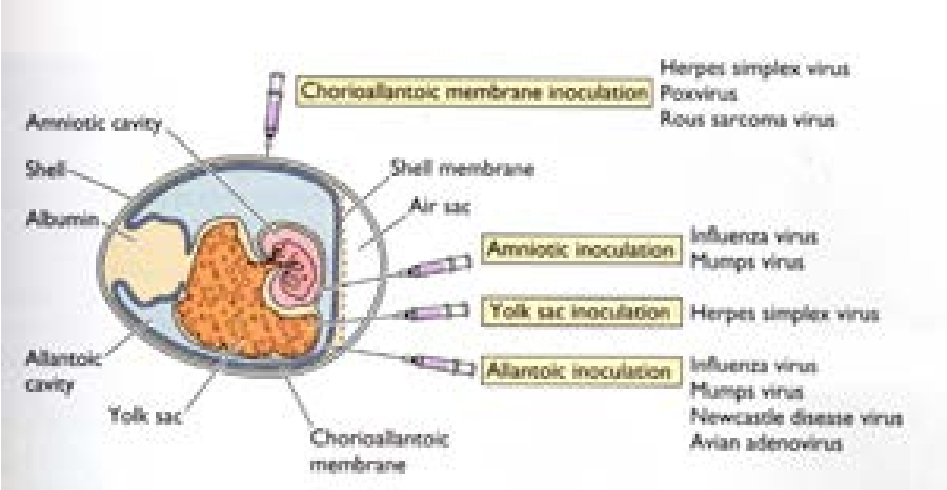
Evaluation in human volunteers

Reverse Genetics



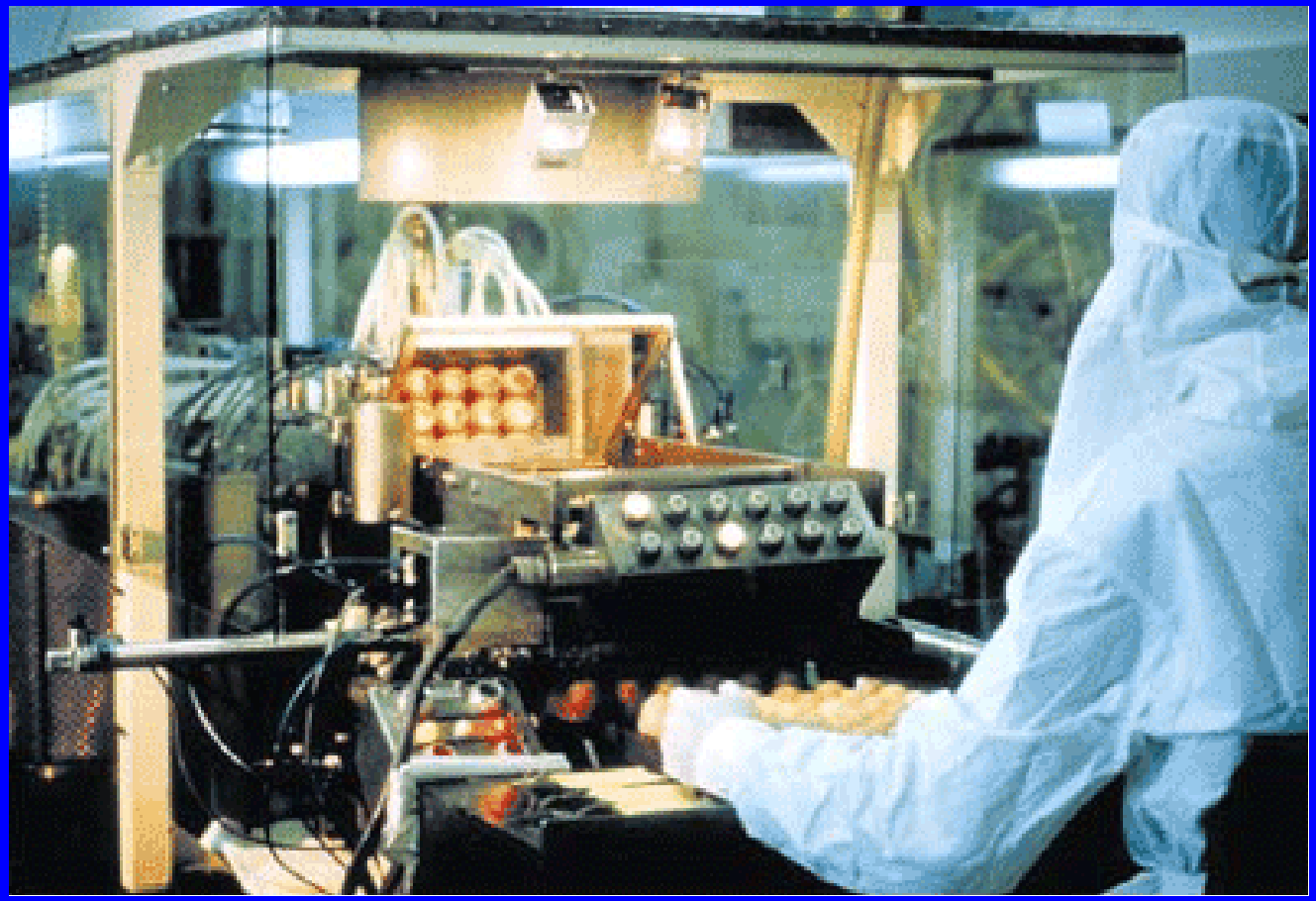
Conventional Methods of Influenza Vaccine Production

Egg propagation



Time consuming, expensive and incompatible with propagating High Path avian influenza strains.

Egg-Based Production of Influenza Vaccine



Technician Working on Egg-Based Production of Influenza Vaccine
Aventis Pasteur MSD/Getty Images

Current Influenza Vaccines

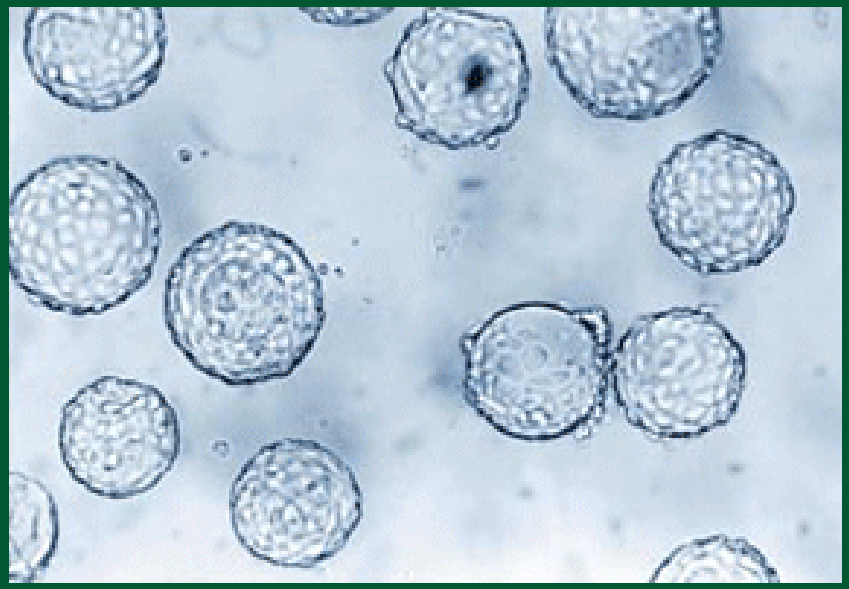
- Safe
- Effectiveness is variable and depends on multiple factors
- Formulated and standardized based on HA content to induce virus neutralizing antibodies
- Egg-based technologies limit rapid response and surge capacity for pandemics

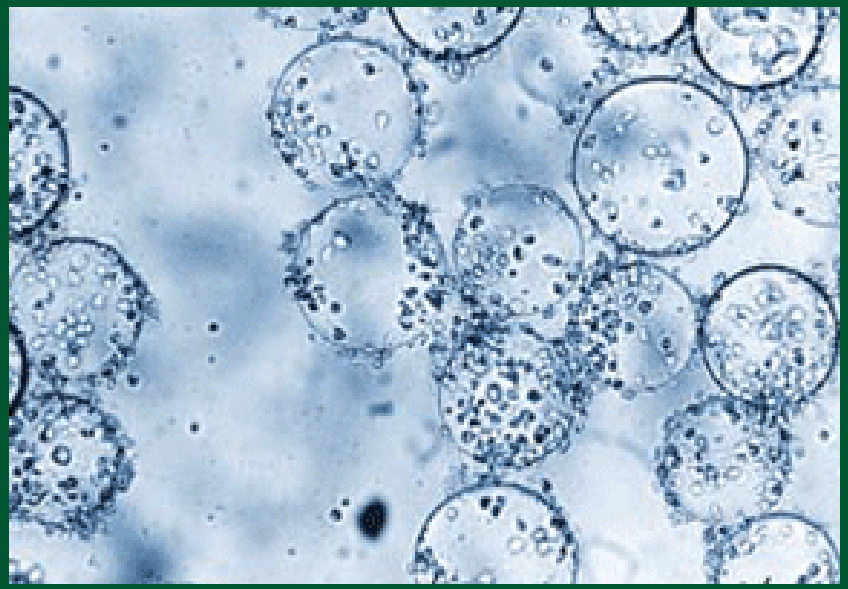
1 dose of Flu vaccine per fertile egg

Would need at least 60 million hens and 4 million roosters to produce enough vaccine each year to immunize the world population. This is about 20 to 30 times the current global vaccine production capacity.

Production of vaccine virus in tissue culture cells poses almost as many problems.

Cell-Culture–Based Production of Influenza Vaccine





Microcarriers with Vero cells are shown before (left) and after (right) infection with influenza virus, Baxter Vaccine.

Live Attenuated Influenza Vaccine

FluMist
Nasal spray;
cold-adapted influenza viruses propagated by infection of cells in culture and manufactured in eggs

Flu Mist Vaccine Credit: MedImmune

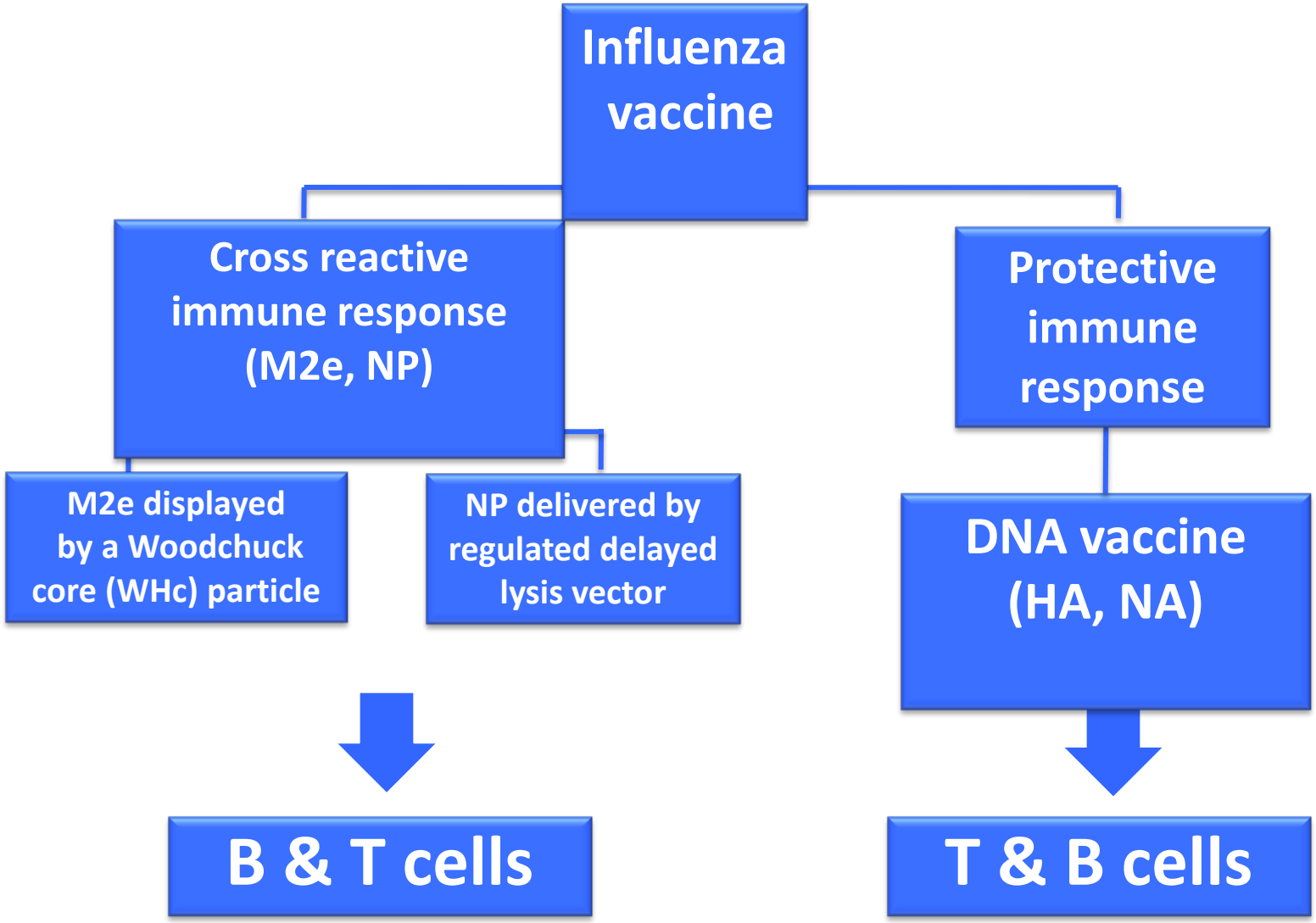
Vaccine induces neutralizing and mucosal antibodies but the extent to which it induces T-cell immunity is not yet known.

Recombinant Attenuated *Salmonella* Vaccine (RASV) Vector

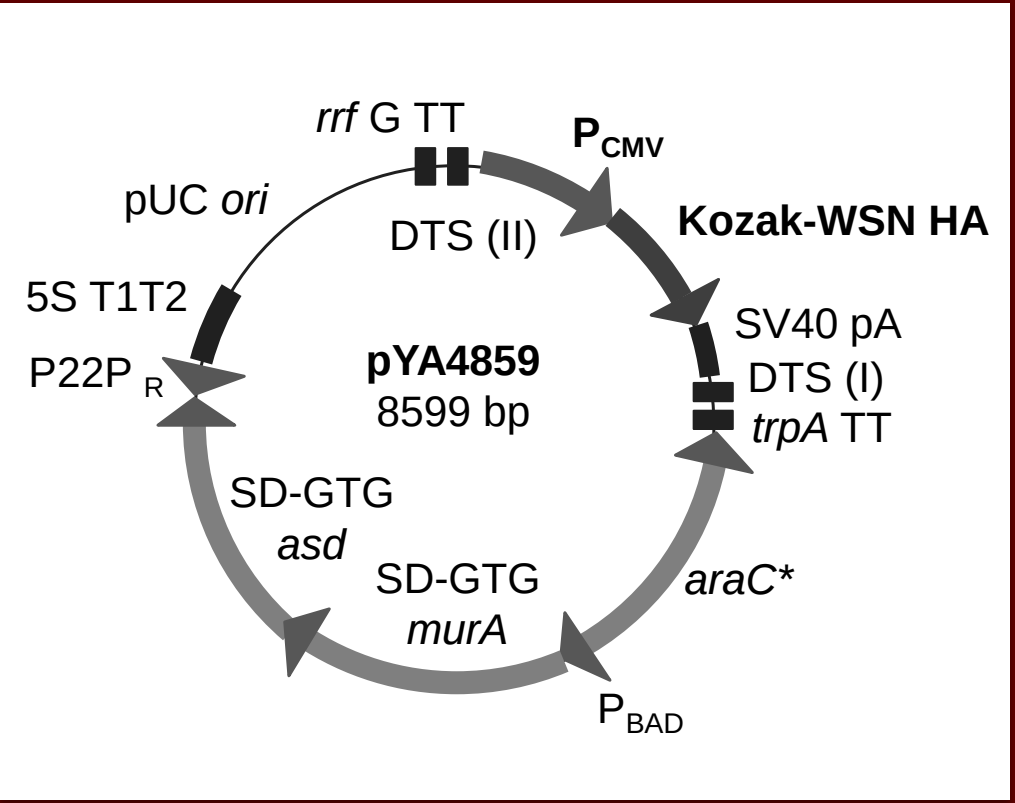
We have now constructed three different recombinant attenuated *Salmonella* vaccines that can be grown in fermentors delivering conserved M2e, NP-HA T-cell epitopes, and a DNA vaccine encoding variable HA antigens to protect humans and poultry against diverse influenza viruses.

Our objective is to induce long-lasting immunity to conserved influenza antigens as well as to the annually variable hemagglutinin (HA) antigens.

The RASV system for delivery of multiple protective antigens to humans (and birds)

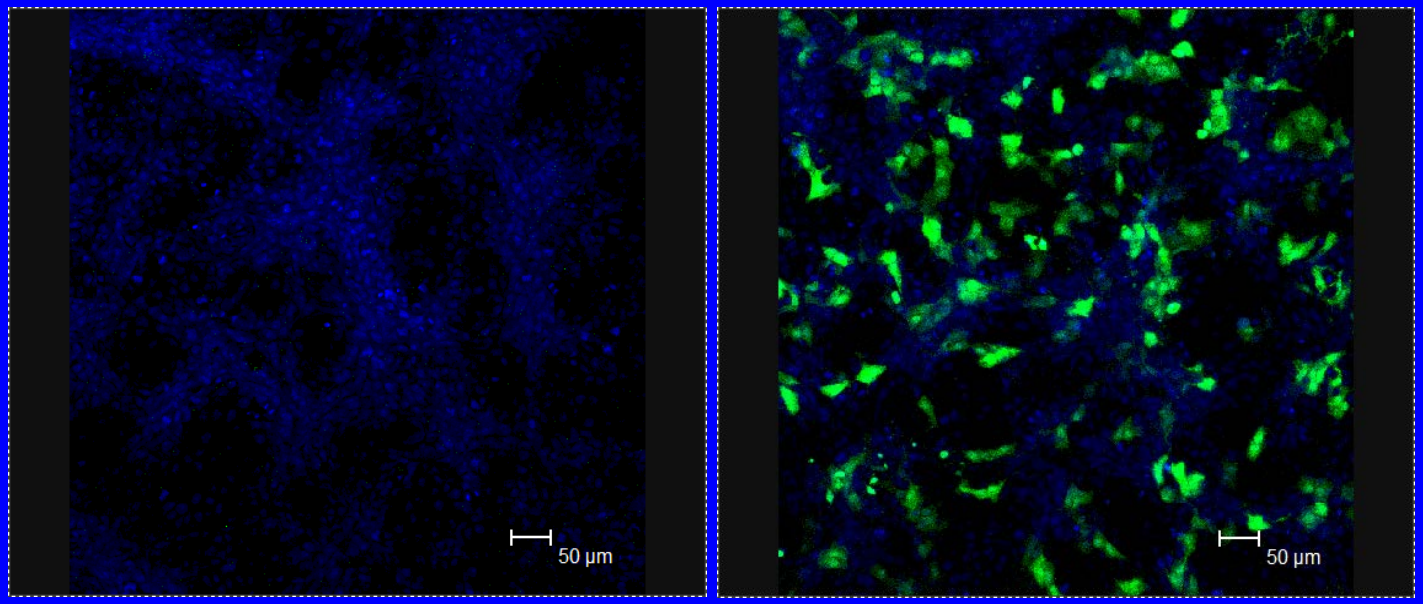


pYA4859 (DNA vaccine vector pYA4545 specifying influenza HA)



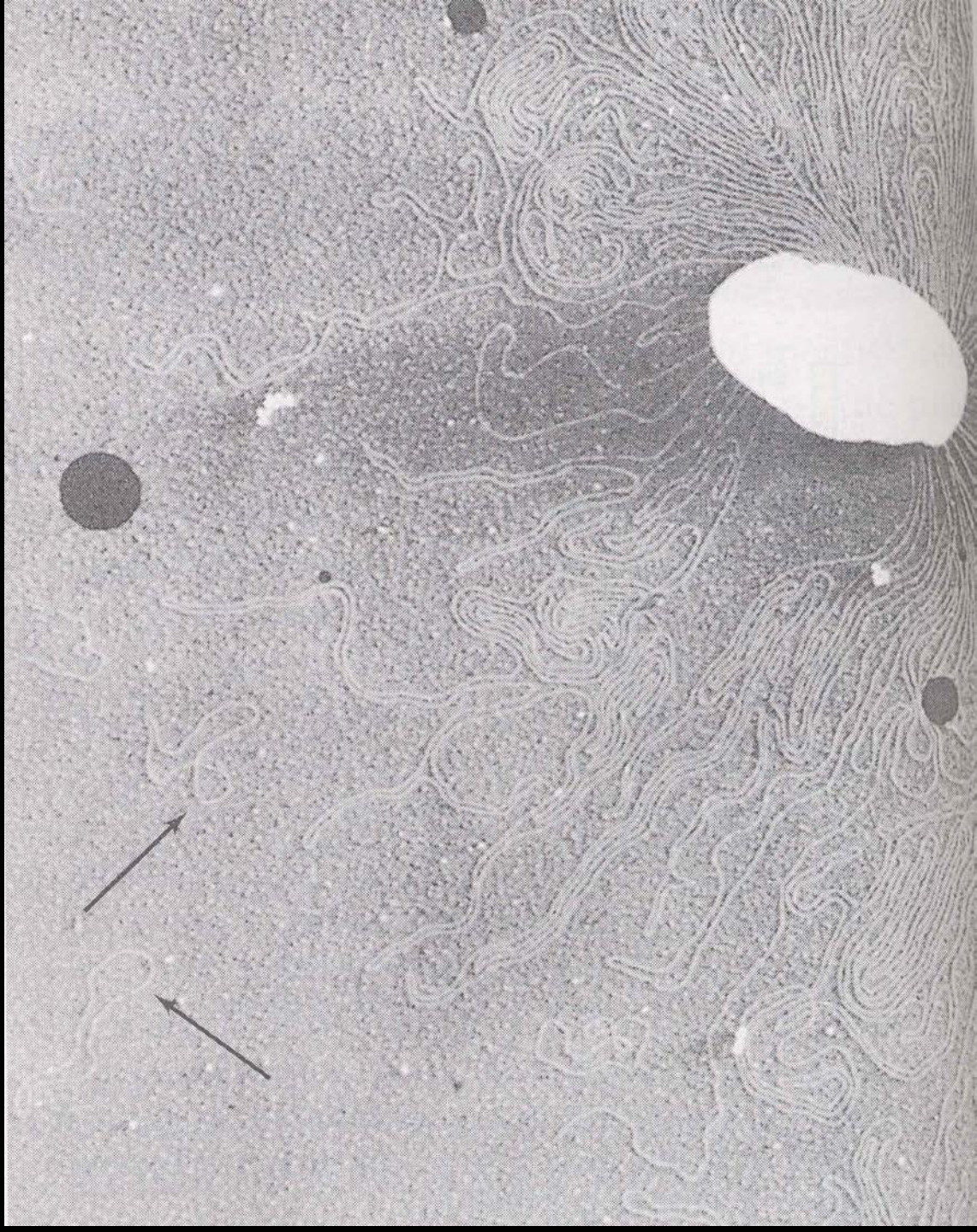
The synthesis of EGFP from pYA4545 harboring EGFP gene in Vero cell line

pYA3650-EGFP (original) **pYA4545-EGFP (improved)**



Transfection of 1 µg DNA vaccine plasmid. TO-PRO-3 iodide (642/661) nucleus staining (Blue) and fluorescence of GFP (green) were visualized 20 hours post-transfection using a confocal microscope (Leica Microsystems Heidelberg GmbH, Objective 20 X).

Salmonella cell lysing to release DNA vaccine vector encoding influenza HA antigen to be synthesized by cells in the immunized human or avian host



Influenza

STOP